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ARSENICAL INJURY THROUGH THE BARK OF FRUIT TREES

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INTRODUCTION

The economic aspects of our interest in the effects of arsenical compounds upon plant life have come to center largely about three conditions that have developed as a result of recent commercial practices, (1) the injury to vegetation by arsenic from smelters, (2) the injury to foliage and fruit by arsenical insecticides, and (3) the possible injury to fruit trees through the bark of the crowns and roots by the corrosive action of arsenicals that have accumulated there as a result of spraying.

In the year 1909 we began at this Station an extensive investigation to determine, so far as possible, all the effects of arsenical compounds upon vegetation. The scope of this work is far-reaching, including the effects of practically all known compounds of arsenic on many different species throughout the plant kingdom. The studies have so far been largely physiological, but it is evident that much chemical work also will be required to solve some of the problems involved.

This paper takes up only one phase of the investigation, but as our work on that is practically complete and it is of immediate interest to orchardists, entomologists, and pathologists, the results are being published in advance of the rest, on which much more work will be required. A preliminary report (10),[1] embodying our first year's work on this subject, was published in 1911.

After fruit trees have been sprayed with improperly prepared arsenical insecticides, it has occasionally been noted that the bark of the twigs has been more or less injured, but in such cases the damage to leaves and fruit has been so severe as to attract much greater attention. This is but natural, for, as the fruit is more resistant to this injury than the leaves, and as the bark is even more resistant than the fruit, it is evident that any arsenical mixture that could be recommended as doing only moderate damage to the leaves would have no visible effect whatever upon the bark.

[1] Reference is made by number to "Literature cited," p. 317-318.

HISTORICAL REVIEW

In 1907, Whipple (11), then Field Horticulturist of the Colorado Experiment Station, in discussing certain rootrots and crownrots that came under his observation, suggested that the more destructive one which "seems to work exclusively in the Ben Davis and Gano * * * may be due to arsenic collecting about the crown of the tree and killing the bark." As he did not carry on extended experiments to prove arsenic to be the cause, and as he suggests that there is some evidence that the disease is infectious, the affected trees being often in groups, this brief note did not arouse much interest.

The next year, however, Headden (5), Chemist of the Colorado Experiment Station, sounded a note of warning to the effect that we would do well to use moderation in applying arsenical compounds to fruit trees for the control of the codling moth and other insect pests and that it would be better, indeed, if these insecticides could be replaced by others not containing arsenic. He offered evidence to show that in Colorado at least much damage was being done by the arsenicals collecting about the crowns of the trees and killing the bark there and eventually the trees themselves, and that, too, in considerable numbers. If we sift from his bulletin the evidence upon which he bases his belief that arsenic is responsible for the killing of the trees in question, we may briefly state it as follows:

1. The trees are dying from injury to the bark on the crowns and bases of the roots, and the cause has not been previously found, though a search for parasites has been made by other members of the Colorado Experiment Station staff.

2. The trees have been sprayed with arsenicals, sometimes in excessive amounts, and these have accumulated in the soil, especially close around the crowns.

3. It is a fact quite generally accepted that solutions of arsenicals are poisonous to plants.

4. Pure water will, to some extent, bring lead arsenate and other so-called insoluble compounds of arsenic into solution, and if the alkali salts frequently found in Colorado soils are added to the water the arsenicals are dissolved to a much greater extent.

5. Arsenic in considerable quantity was found in the soil under the trees and in the injured trees themselves.

6. A tree known to have been badly injured by sodium arsenite poured into an irrigation ditch near it presented symptoms similar in some important respects to the many dying trees under discussion.

In a later bulletin (6) he strengthens some of these points of evidence, but emphasizes the fact that, while the alkali salts make the arsenicals more soluble and are doubtless of importance in some orchards, they are not at all necessary to bring a portion of the arsenic into solution, and in

some orchards where they are not present to any considerable extent in the soil the trees show corroded crowns and an abundance of arsenic in the wood. In the same bulletin he also cites (p. 19) an observation that is of especial interest in this connection and is as follows:

> Dr. S. M. Bradbury, Horticultural Inspector of Mesa County for eight years, makes the following statement, which is used with his permission. "Bands had been used in a certain orchard and on adopting the lime, sal soda and arsenic spray these bands were left on the trees, some of them for two or three years. Some of these trees died and on examining the bands we found that the bark beneath the bands had been entirely eaten up. The lime and arsenic had collected under the bands and destroyed the bark."

While Headden does not claim that this evidence is proof positive that arsenical compounds are responsible for the death of the trees, he is evidently thoroughly convinced that such is the case. His line of argument presents some points of weakness. He does not prove that trees that are uninjured, or not obviously injured, may not contain as much arsenic as the injured ones. He does not prove that the arsenic was taken through the corroded crowns or that, though poisonous if absorbed through the feeding roots, it can attack the cork-protected bark. He does not prove that arsenic in small quantities is injurious to fruit trees nor does he establish the point of concentration above which it becomes injurious. He does not establish points of difference between the injury in question and that found in other States on trees planted in virgin soil to which no arsenic has ever been applied for any purpose and where, therefore, arsenic can not be a factor in causing the injury. While some vain search for parasitic fungi and bacteria has been made at the Colorado station, their absence is very difficult to prove conclusively, as testified to by the many unsuccessful early attempts to discover the crowngall organism.

At least two investigators dissent from Headden's conclusions. Ball, of the Utah Experiment Station, would attribute the cause directly to alkali and seepage water, and Grossenbacher, formerly of the New York Experiment Station, shows some points of similarity to a form of crown-rot prevalent in New York and elsewhere which he attributes to winter injury. These discussions will be reviewed later in this paper (see p. 316).

This very suggestive work of Headden's, while not accepted by all as conclusive, has made a strong impression upon many horticulturists, entomologists, and plant pathologists over the country and has pointed out the necessity for a study of the physiological aspects of the questions. It is highly important to determine beyond all dispute whether or not trees are injured at the crowns by arsenical spray mixtures and, if so, the conditions upon which this injury is dependent. We have, therefore, treated numerous branches of various sizes, trunks, crowns, and roots with different arsenical compounds, including those used as insecticides, to study the injury to bark and wood if any should be produced.

EXPERIMENTAL METHODS

We have endeavored, among other things, to reproduce as nearly as possible the conditions obtaining about the crowns of the trees as a result of spraying, especially with reference to the presence of the arsenicals in immediate contact with the bark, and the moisture necessary for bringing them into solution. When crowns were to be treated, this was accomplished by carefully removing the earth without injuring the bark, applying the arsenical, and replacing the earth. The natural precipitation and the regular orchard irrigation were depended upon to furnish the necessary moisture. This method, while simulating closely the conditions brought about by orchard spraying, is obviously cumbersome. It is slow of application, frequent examination to note the course of the injury is difficult, and as each tree could be used for only a single treatment a good-sized orchard would have been required to furnish trees for all the tests we have made, the cost of which would have been prohibitive. We therefore made considerable use of trunks and branches.

When the trunks were treated, this was done by a method shown us at the Utah Experiment Station by Dr. E. D. Ball, to whom we are indebted for the suggestion. A funnel of white rubber sheeting was constructed about the trunk and sealed to it with tree tanglefoot (Pl. 79). Inside this, the arsenical was placed, distilled water was added, and the whole covered with a hood of rubber sheeting sealed to the trunk above and loosely overhanging the funnel. This served to keep out rain water and permitted free access of air. This method is open to most of the objections mentioned above and, furthermore, the rough, shaggy bark of the trunk makes it very difficult to keep the funnels from leaking. If one attempts to smooth this up by scraping there is considerable danger of making unnatural breaks in the corky bark. It is not unlikely that Dr. Ball used a more satisfactory wax than tree tanglefoot. As a matter of fact we used this method in only nine cases—those reported in Table VIII.

The great majority of the tests recorded in this paper were made on branches of various sizes, and the chemical was held in contact with them by means of a bandage of absorbent cotton protected by a wrapping of surgeon's gauze, the whole being wet with distilled water. As the orchard had never been sprayed, it seemed desirable at the outset to prevent the escape of any of the arsenic from the bandage to the soil so that any injury that might occur could not possibly be attributed to absorption through the feeding roots. This end was attained in the early experiments by selecting branches that showed a decided sag at some point and placing the bandage at the lowest part. A metal box was hung close under the bandage to catch any drip produced by rains. Later we tried wrapping the bandages in a dental rubber dam to prevent the drip, and omitting the boxes (Pl. 80). In repeated tests the two methods gave results almost identical, so the boxes were discarded as they were more cumbersome,

required more frequent watering, and were applicable only to branches having the right kind of sag, which in time became difficult to find.

In those bandages that were not covered with rubber the conditions of moisture and aeration were closely similar to those often found about the crowns a few inches beneath the surface of the ground. They were wet with distilled water every day, but owing to our atmospheric conditions, that favor rapid evaporation, they were, as a rule, nearly or quite dry an hour or more before the daily watering. Those that were wrapped in rubber were wet continuously and the aeration was not so good, but many comparative tests gave results almost identical by the two methods, or as nearly identical as the duplicates were by either method alone. We were, therefore, led to believe that excessive moisture and imperfect aeration did not materially influence the injury, which had usually begun in a few days and was complete within two or three weeks from the time of treatment and probably before the bark would make any abnormal growth due to lack of air or excessive moisture.

In the few cases where roots a little distance from the crown were treated it was by the same methods as described for the branches. Root treatments required so much labor, however, that only enough were made to assure us that the susceptibility of roots is not greatly different from that of branches. This work was further complicated by the difficulty of finding roots of suitable size that did not bear rootlets which, when broken off by digging, presented unnatural openings for the entrance of arsenical solutions.

This work was all carried on in the college orchard at Bozeman, where some branches could be injured and removed without serious damage to the remainder and where, by good fortune for this work, a few rows of trees were to be removed entirely and were available for the most severe treatment of crowns, trunks, and large branches.

The tests were made almost entirely upon apple trees, though pear and plum were tried in a few cases. Where the latter were used, they are specifically mentioned; otherwise, the results described in the following pages apply entirely to the apple.

ARSENICAL COMPOUNDS USED

In carrying on work of this kind much difficulty is encountered in determining the exact composition of arsenical compounds regularly put upon the market or even in getting made to order all the different compounds wanted, free from mixture with other arsenicals. There is a great tendency on the part of insecticide companies to prepare mixtures, especially of the diplumbic and triplumbic lead arsenates. We were fortunate, however, in securing, on special order from the J. T. Baker Chemical Co., of Phillipsburg, N. J., most of the more important arsenicals apparently quite pure.

There exists much confusion of names for the ortho lead arsenates. There seem to be only two that are commonly manufactured as insecticides. These are the diplumbic and the triplumbic lead arsenates. The diplumbic is also called "monoplumbic," "acid," and even "pyro." The triplumbic is variously termed "neutral," "normal," "basic," and "ortho" lead arsenate.

Following is a list of the chemicals used, with the percentage of arsenic that each contained as determined by the chemistry department of this Station. The arsenic was calculated on a moisture-free basis.

	Per cent
Arsenic acid, Merck	51. 10
(Merck & Co., New York and Darmstadt.)	
Ammonium arsenate, Merck	45. 20
Ammonium arsenite, Merck	68. 45
Arsenic trisulphid (yellow), Baker	59. 10
(J. T. Baker Chem. Co., Phillipsburg, N. J.)	
Arsenic trisulphid (yellow), Merck	26. 12
Arsenic trioxid, Baker	73. 10
Arsenic trioxid, Merck	75. 40
Calcium arsenite, Baker	51. 20
Calcium arsenite, Merck	44. 78
Copper aceto arsenite (Paris green), Ansbacher	42. 42
(Ansbacher Insecticide Co., Inc., New York.)	
Copper aceto arsenite (Paris green), Baker	42. 85
Iron arsenate, Merck (a mixture of ferrous and ferric)	38. 40
Lead arsenate, diplumbic, Baker	21. 31
Lead arsenate, triplumbic, Baker	16. 88
Lead arsenate, mixture, Grasselli	19. 21
(Grasselli Chemical Co., Cleveland, Ohio.)	
Lead arsenate, diplumbic, Grasselli	21. 13
Lead arsenate, triplumbic, Grasselli	14. 60
Lead arsenate, "technical," mixture, Merck	19. 25
Lead arsenate, mixture, Swift	20. 19
(Merrimac Chemical Co., Boston, Mass.)	
Lead arsenate, diplumbic, Swift	
Potassium arsenate, Merck	41. 32
Potassium arsenite, Merck	49. 70
Sodium arsenate, Merck	24. 75
Sodium arsenite, Merck	58. 15
Zinc arsenite, mixture, Baker	53. 00
Zinc arsenite, meta, Baker	52. 75
Zinc arsenite, ortho, Baker	31. 90
Zinc arsenite, pyro, Baker	41. 33
Zinc arsenite, Merck	35. 10
Zinc arsenite, ortho, California Spray Co.)	32. 14
(California Spray Co., Watsonville, Cal.)	
Zinc arsenite, "Ortho 40," California Spray Co	24. 38

NATURAL PROTECTION OF THE BARK

It has long been known that the outer portion of the bark of many trees is made up of a layer of cork varying in thickness from few to many cells. On apple trees (*Malus sylvestris*) this forms a well-developed

coating on all parts except the leaves and the youngest stems and roots. This layer of cork is well formed on the young shoots when they go into their first winter and persists thereafter. It forms a layer that is smooth and for the most part fairly even in thickness until the limbs and trunks reach a considerable size. As the limb enlarges inside this layer, new cork cells are added from within. The older cork cells on the outside slough off to some extent, but, as a rule, no definite breaks are found until the natural formation of rough bark begins. This takes place when the trunk or the limb is from 2 to 6 inches in diameter, depending upon variety and environmental conditions.

The well-known roughened condition of the bark is brought about by rifts or splits forming in the outer corky layer and the tissues lying just beneath it (Pl. 81, *A*). These often begin at the intersection of branches, in crotches, and about old wounds and other irregularities, and gradually extend over the entire trunk and the larger limbs (Pl. 81, *B*). While these cracks form more or less gradually and cork is produced later at their inner extremities, there is often a period when the living cells of the bark are wholly unprotected in places by the cork cells. Thus, it happens that while it might seem at first thought that the heavy, rough bark of trunks and crowns is a thicker and better protection than is found upon the smaller branches, such in reality is not the case; for while the cork of this rough bark is in some places very thick, in others it is quite broken through. Table I records a series of observations made in the late fall of 1910 upon the thickness of the cork on branches of different ages. Further examinations made in July, 1915, indicate that there is very little difference between the dormant and active periods.

TABLE I.—*Number of cells in periderm of smooth bark and in the furrows of rough bark of apple trees*

Year's growth.	Character of bark.	Diameter.	Cells in cork layer.	Character of bark.	Diameter.	Cells in cork layer.	Character of bark.	Diameter.	Cells in cork layer.
		Inches.			*Inches.*			*Inches.*	
1910.....	Smooth[a]..	1/4	3–5	Smooth[c]..	1/32	1–3	Smooth[e]..	1/32	2–5
1909.....	...do.....	1/4	2–5	...do.....	1/32	4–6	...do.....	1/32	2–5
1908.....	...do.....	1/32	3–5	...do.....	3/32	3–7	...do.....	1/4	2–5
1907.....	...do.....	3/32	4–6	...do.....	1/32	3–7	...do.....	3/32	3–7
1906.....	...do.....	3/16	4–8	...do.....	1/2	4–7	...do.....	3/16	2–7
1905.....	...do.....	5/32	4–9	...do.....	3/4	3–7	...do.....	5/32	4–8
1904.....	...do.....	3/4	4–8	...do.....	7/8	2–8	...do.....	7/8	3–7
1903.....	.....	.....	.....	...do.....	1 1/8	5–7	.....	.....	.....
1902.....	Smooth[a]..	7/8	3–8	...do.....	1 3/8	1–8	.....	.....	.....
1900.....	...do.....	1 1/8	4–10	.....	.....	.....	.....	.....	.....
1899.....	...do.....	1 1/4	4–9	.....	.....	.....	.....	.....	.....
1896.....	...do.....	2 1/4	4–9	.....	.....	.....	.....	.....	.....
.....	...do.....	4	4–many	Smooth[d]..	4	4–many	Smooth[e]..	4	3–many
.....	Rough[b]...	6	2–many	Rough[d]...	6	4–many	Rough[f]...	6	6–many
.....	...do.....	8	5–many	.....	.....	.....	...do[g].....	8	3–many
.....	...do.....	12	1–many	.....	.....	.....	.....	.....	.....

[a] Okabena.
[b] Transcendent crab.
[c] Hibernal.
[d] Liebe.
[e] Wealthy.
[f] Oldenburg.
[g] Orange.

OPENINGS THROUGH THE CORK LAYER

Even on the smooth bark, where the layer of cork is for the most part fairly even, the protection offered is not perfect. At the time the periderm is forming, the lenticels are forming also. On apple bark these are quite numerous and of varying sizes (Pl. 82, *A*, *B*). While they have not been studied with such thoroughness as those on some other woody plants, it is well established that they have numerous, though rather small, intercellular spaces and permit free access of air for several years. As the stem grows in diameter, they enlarge very much and on some varieties they divide into groups. From their appearance under the microscope (Pl. 83, *A*, *B*) we would judge that some of them at least remain functional for several years, sometimes until nearly the time when the bark becomes furrowed and roughened. While the lenticels are known to admit air, the extent to which they will admit liquids can be determined only by experiment. On the smooth bark the lenticels are the only natural openings after the disappearance of the stomata, but artificial openings can easily be made and do frequently occur as a result of hail, insects, and various orcharding operations. The rough bark, while less easily broken by external agencies, is frequently cracked by the growth within, as described above. The roots also, after reaching a diameter of ¼ to ½ inch, have lenticels in varying sizes and numbers, which for the purposes of this investigation are not essentially different from those on the aerial portions (Pl. 82, *C*).

ARSENICAL INJURY THROUGH THE SMOOTH BARK

When arsenical compounds of almost any kind are applied to the branches by the methods described above, if the quantity is sufficient, more or less injury follows. In many cases the natural protection of the cork and the penetrability of the lenticels and wounds are clearly shown.

CHARACTER OF INJURY

Roughly speaking, we may say that the various compounds of arsenic produce the same symptoms of injury, varying only in degree.—that is, a given amount of injury by one compound is very much like the same amount produced by another. Calcium-arsenite injury in differing severity will therefore be described; and let it suffice for a general description of arsenical injury through the smooth bark, keeping in mind only that with us some of the milder compounds like ortho zinc arsenite and iron arsenate have never produced the severest injury.

When the bark is unwounded and the injury is only slight, it is not visible to the eye for several days, usually a week or more, depending somewhat upon the temperature, and perhaps other conditions. Then it may be noticed that close around certain lenticels is a small indefinite zone which is slightly darker in color on the surface than the surround-

ing bark. This forms a tiny spot, with the lenticel in the center (Pl. 83, *C*). If the affected tissues are cut into with a knife, they are found to be darkest near the surface; but the color grades imperceptibly into that of the healthy bark a millimeter or two below the surface. Each day now for about a week or two the spot becomes a little darker externally and internally and increases a little in size. It then becomes sharp in outline, as a rule, and quite definitely sunken. Two or three weeks after treatment, if made during the growing season, most of the spots cease to grow in size. They are then very dark brown, almost black, externally and much sunken, and a crack begins to form at the boundary between the injured tissues and the healthy ones. These spots vary in size from one to several millimeters and often anastomose into irregular dark patches. As a rule, the spots are larger on the under side of a horizontal branch than on the upper. The difference, however, is somewhat less noticeable if the bandages are covered with a rubber dam and thereby kept evenly wet. In a spot 5 mm. in diameter the tissues are killed and colored a very dark brown to the cambium and the youngest wood is sometimes affected also. In a spot 10 mm. in diameter the bark is discolored to a depth of one to several millimeters and there is considerable injury to the underlying wood. The affected bark is not friable at this time, but tough and leathery. Not all the lenticels are affected, as a rule; sometimes only a few and sometimes many, from which we may judge that some of them are closed at times.

If the action of the chemical is more severe, many lenticel spots are usually found anastomosed and the dead patch thus formed may girdle the limb under the bandage. From this dead patch one or more streaks may run up and down the limb. These vary in length and diameter, and their size may be used to indicate the severity of the attack. In extreme cases they are several feet long and kill some of the side branches. These streaks, like the spots, are at first indefinite and later become nearly black and sunken. The wood under the dead bark is also discolored, varying from a light coffee-brown to nearly black. This discoloration may invade only the wood next to the cambium, but often it extends to the pith.

When a wound is made through the outer corky layer just before the chemical is applied, the tendency to form streaks is much more marked than when the absorption is through lenticels. There will appear around the wound more or less discoloration and in mild cases the injury stops here. Sometimes near-by lenticels will show spots also. There is a strong tendency, however, to form a streak running up and down from the wound (Pl. 84). As a rule, it extends farther upward than downward; but the reverse is sometimes true. Sometimes the whole branch above the bandage is killed, and only a streak runs down. Sometimes the streaks run a spiral course, and if there are two wounds on opposite sides of the branch the two streaks from them keep on opposite sides and

run a parallel course even though both are spiral. As a rule, the streaks are continuous, but occasionally they are intermittent, disappearing from view and reappearing higher up. In all such cases examined, however, the streak in the wood was continuous, the span of sound tissue being only in the bark. These streaks may show almost every proportional variation in length and width. There is a strong tendency for the streaks that run down to be terminated by intersection with a larger branch, but there are frequent exceptions.

The leaves on some part or all of the affected branch may also show more or less injury. The first symptoms may be apparent as early as the first or second day after treatment. The leaves at first lose their normal luster and look dull and a little lighter green. This condition may affect the entire leaf, but often only spots and irregular areas. In bad cases there is considerable wilting. Soon the spots so affected turn brown and become dry and crisp. It is a notable fact that the leaves so affected are practically always directly touched by a discolored streak or area. A twig touched by such a streak may have every leaf killed, while on the opposite side of the limb, even if it is only a half inch in diameter, a twig will have leaves with no visible trace of injury.

In studying the symptoms of injury through the smooth bark in many different cases, one is impressed by the great uniformity of certain general features and the great variety of differences in minor details. The uniform and constant symptom is localized discoloration of bark and wood, beginning as an indefinite discoloration and ending after a period of a few weeks as a sharply defined, dark, sunken area. If the absorption of arsenic is slight, the discolored region does not extend much farther longitudinally than transversely; but if the absorption is considerable, as through a wound, a long and relatively narrow streak is produced. Beyond these general features there is little uniformity, the relative length and breadth of the streaks, their relative length above and below the point of absorption, and their branching at intersections, with side branches presenting an endless variety of detail.

A feature of this work that has given us constant trouble is the frequent failure of duplicate and triplicate tests to check with each other. When two or three applications were made under conditions apparently identical in every respect, the resulting injury was not always the same in extent and character. In the majority of cases the results were nearly enough alike for practical purposes (Table VI); but all too frequently duplicate and triplicate tests gave widely different results, as may be seen, for example, in Tables IV and V. These differences were most marked in limbs that had been wounded just before treatment and gave much less trouble in those that were unwounded. As a result of this erratic behavior, most of the tests were repeated over and over again for confirmation; and the reader is warned against drawing hasty and definite conclusions

from a comparison of any two treatments. It is possible that a thorough anatomical and histological study of branches that showed wide differences under identical treatment might have revealed the explanation, but the importance of the question did not seem to warrant the expenditure of time.

One of the most striking facts connected with this type of injury is the self-limitation of the streaks. At the end of two weeks they have practically always run their course, unless the treatment was given in late fall or winter. For a week or two longer the margins continue to grow sharper and the dead bark becomes more sunken, but almost never is there any material lengthening or broadening. The explanation of this early limitation is not clear to us. At first it seemed possible that the chemicals used contained some soluble impurity which became entirely absorbed during the first week, after which there was little injury; but this idea has been wholly abandoned. We have used chemicals made up with especial care as to purity, and we have washed them in many changes of distilled water, but the results in this respect were the same (see p. 302). Also, we get this same limited course from bandages kept saturated with the soluble arsenicals, such as sodium arsenite and arsenic acid. We have established quite conclusively that this discolored wood and bark conducts the solutions very poorly; this probably has much to do with the phenomenon. However, it must be remembered that liquids pass rapidly up the xylem ducts and some would be expected to reach the leaves at the tip of the branch very quickly before any visible change takes place. That some arsenic does actually reach distant parts that continue to appear healthy has been established by chemical tests made by the Station chemist. It seems quite probable that by the action of the tissues through which the chemicals pass some of the arsenic is withdrawn from solution, enough so that what passes on is too weak to cause the complete death of the cells or a noticeable discoloration of the ducts.

To determine further the relation of the absorption of solutions through wounds to the killing of tissues by the solutions absorbed, the following experiment was conducted: Three wounded limbs were each treated with 10 gm. of calcium arsenite, three others with 10 gm. of calcium arsenite and 1 gm. of methylene blue, and three others with 1 gm. of methylene blue alone. No injury was produced by the stain alone, and the injury by the arsenical alone was about like that with the arsenical plus the stain. When examined a month after treatment, it was found that where the stain was used alone it made relatively narrow streaks up and down from the wound. These streaks were not visible externally and affected only the youngest of the inner bark and extended into the wood for a depth of one to three annual rings. They were of an intense blue color near the wound

and became paler and narrower until they disappeared. Where the stain was used with the arsenite, it was the same in character as where used alone, but was always much narrower than the dead streak surrounding it and usually shorter. Also, several side limbs were more or less injured by the arsenical, but contained no visible trace of the blue. In no case did the blue extend into living wood or bark. These results would suggest that the solutions absorbed by the wounds are not carried very far beyond the dead portions.

INJURY AS AFFECTED BY CHARACTER OF WOUNDS

Some questions arise as to the relative importance of wounds of different character. Are small abrasions sufficient to make injury possible? Does a wound just through the periderm admit the arsenic as freely as one extending to the cambium or into the sapwood? When do wounds like those made by pruning become sufficiently closed to keep out the solution?

From a study of Table II it is evident that even a very slight rupture of the periderm is sufficient to make possible severe injury to the limb if the arsenical is applied continuously in a moist state (10, p. 86). So far as these few tests go, we may infer, however, that the chemical is absorbed less rapidly if the wound is superficial than if it extends to the cambium. If, however, the slower injury, like that through the superficial wound in No. 3, results in a girdling of the limb, it is just as surely fatal to the portion above as though absorption were more rapid. In comparing the injury through wounds made by cutting off twigs and branches with that through wounds made by cutting out a small piece of bark to the cambium, we find that sometimes the injury is worse through the former and sometimes through the latter. On an average, there is not a great deal of difference.

TABLE II.—*Results of wounding and treating branches of Early Strawberry apple on August 21, 1911, with 5 gm. of Baker's copper aceto arsenite, to show the relative injury through wounds of different size and character*

No.	Diameter of treated branch.	Wound under bandage.	Extent and character of injury.
	Inch.		
1	¾	Branch ⅜ inch cut off	Streak half around branch runs down from wound 1 foot and up 3 feet to the tip.
2	⅜	One-fourth inch square of bark removed to cambium.	Streak half around branch runs down 7 inches from wound and up 4 feet nearly to the tip.
3	⅜	One-fourth inch square of outer cork removed.	Streak half around branch runs down from wound 2 inches and up 3 inches.
4	⅜	Transverse cut through bark to cambium on one side.	Girdled under bandage, except for a small strip on side opposite wound.
5	⅜	Radial cut 1 inch long through bark to cambium.	Streak from wound runs down 7 inches and up 20 inches.
6	⅜	Five fine needle pricks through bark.	Entirely girdled under bandage. Streak runs down 5 inches to main limb and up nearly to tip, 2½ feet.
7	⅜	Old pruning wound	Injury extends 1 inch up and 1 inch down from wound with little at the sides. A few lenticel spots.
8	⅜	Unwounded	Numerous lenticel spots ¼ inch or less in diameter.
9	⅜	.....do	Numerous lenticel spots $\frac{1}{16}$ inch or less in diameter. Two small spots around latent buds.

Table III shows quite conclusively that the injury through wounds of a few months or older is much less than through freshly made wounds. This has been tested at different times on branches other than those listed in this table, and it has been our general observation that while there is sometimes more or less darkening of the wood through wounds made several months or even two or three years before treatment, there is rarely any serious injury to bark or leaves. A few exceptions have been observed.

TABLE III.—*Results of treating branches of apple on July 15, 1912, with 25 gm. of Baker's calcium arsenite, to show the relative injury through wounds of different age and character*

No.	Diameter of treated branch.	Wounds under bandage.	Extent and character of injury.
	Inches.		
1	1	Branch 1 inch diameter cut off 4 years ago.	A few lenticel spots.
2	1¼	Branch cut off 4 years ago. Partly healed.	Slight discoloration on under side of branch under bandage.
3	1⅛	Branch 1⅛ inches diameter cut off 3 years ago and completely healed over.	Branch under bandage almost girdled by anastomosing lenticel spots. No special injury around wound.
4	2¼	Branch 1¼ inches diameter cut off 3 years ago. Partly healed.	Practically no injury.
5	1¼	Bark peeled off ¼ by 1¼ inches 3 years ago.	Indefinite streaking to partly killed side branch 2½ feet above bandage. A few lenticel spots.
6	1¼	Branch 1 inch diameter cut off 2 years ago.	No visible injury.
7	2¼	Branch ¾ inch diameter cut off in winter of 1912.	A few small lenticel spots under bandage.
8	1½	Branch cut off in late winter of 1912..	Nearly girdled under bandage. No special injury around wound.
9	2¼	Branch ¾ inch diameter cut off 2 months ago.	No visible injury.
10	¾	Two twigs cut off 2 days before treatment.	Branch killed above and under bandage. Sunken streak runs from each wound to tip.
11	1¼	Small branch cut off and notch cut to cambium 2 days before treatment.	Branch killed above and under bandage. Sunken streak runs to tip, 4 feet.
12	1	Twig and small branch cut off just before treatment.	Branch killed above and under bandage. Sunken streak runs to tip, 4½ feet.
13	¾	Twig cut off and notch cut to cambium just before treatment.	Branch killed above and under bandage. Streak runs down 1 foot, injuring 3 side branches.

PROTECTION OF WOUNDS BY PAINTING

It was suggested in our preliminary report (10) that white-lead paint applied to wounds, even though they were freshly made and the arsenical was applied before the paint was dry, would offer some measure of protection. This suggestion was based on very few trials, which left it in need of further investigation. Paint was therefore applied to wounds of various sizes and ages and these were afterwards treated with copper aceto arsenite or, in a few cases, calcium arsenite. More than 50 painted wounds were so treated. In some cases the chemical was applied as soon as the paint was put on and in others the paint was allowed to dry for different lengths of time. The wounds were made either by cutting off a small twig or by cutting a notch through the bark to the cambium.

Seven wounded limbs were painted with white-lead paint and immediately treated with copper aceto arsenite. There was considerable

injury in every case. The least was a streak running up 11 inches and down 3 inches. In the worst injury the entire branch above the bandage was killed, and a dead streak ran down 10 inches. Between these two extremes there were all gradations. In comparison with the injury through unpainted wounds, it was clear that the paint had in some cases given a partial protection, but in others little or none.

A few wounds were left unpainted in the center to see whether the arsenical entering through the wood at that point would work outward and cause an injury to the bark. The failure of the paint to protect when applied to the entire cut surface leaves the result doubtful in these cases.

Twenty-one other wounds of a similar nature were painted with white-lead paint as soon as made, and, after an interval for drying, 10 gm. of copper aceto arsenite was applied to each. On four of these the paint was allowed to dry for 6 hours before treatment, on six others it was allowed to dry for 7 hours, and on the remaining eleven 24 hours. The work was done in dry, sunny weather; and even in 6 hours the paint showed considerable evidence of drying. In 24 hours it seemed quite dry, though not very hard. In general, the paint under these conditions reduced the amount of injury very materially as compared with unpainted wounds similarly treated, and yet in nearly every case streaks several inches long were produced and in four cases the entire branch above the bandage was killed. In three of these four cases the paint had dried for 7 hours and in the other 24 hours.

Eleven branches varying in size from 1½ to 3⅛ inches were cut off several inches from the large limbs on which they grew, so that long stubs were left. These stubs were painted on the cut ends and for about 2 inches back on the uninjured bark. Some were given one coat and some two, with an interval of 24 hours for drying after each coat. The end of each stub was treated with 25 gm. of Baker's calcium arsenite, the bandage being applied in the form of a cap and covered with a dental rubber dam. In some cases, especially where two coats of paint had been applied, the action of the chemical was considerably lessened, but in the majority, including some with two coats of paint, the injury was excessive, running down the stub and into other parts of the tree. In no case was the protection complete.

Nine other stubs were similarly treated with spar varnish, but this offered no better protection than the paint.

It should be noted in this connection that in these large stubs the pressure of sap was so great as to form blisters under the paint and varnish; and these often broke open, which would easily account for the injury. This condition, however, did not occur on the wounds on smaller limbs.

If it were a matter of great practical or scientific importance, it is possible that a covering could be found that would give better protection than either white-lead paint or varnish.

RELATIVE INJURY TO BRANCHES OF DIFFERENT SIZES

As it was difficult to find enough branches of exactly the same size to carry on this entire investigation and as there is some purely scientific interest in knowing whether smaller branches are more or less susceptible than larger ones, especially through the lenticels, a series of tests was made on limbs and branches of as widely varying sizes as could be found without including any having rough bark.

Twelve unwounded branches were treated with 10 gm. each of Baker's calcium arsenite. These varied in size from ¼ inch to 2½ inches. Of the three that were only ¼ inch in diameter, two were killed above the bandage, and the other was nearly girdled under the bandage but uninjured above and below. The rest all showed anastomosing lenticel spots. On the branches less than 1 inch in diameter the enlargement of these spots had resulted in a complete girdling, and in some cases in streaks several inches long. On the larger branches, of which there were six, the spots were a little more distinct and about alike on all, with no streak above or below the bandage.

If, then, the branches are unwounded, we find that in those between ½ inch and 1 inch in diameter there is no consistent difference in susceptibility dependent upon size. There is sometimes found a considerable variation on smaller-sized branches even on the same tree; but, between the limits just named, size seems to be an unimportant factor. Unless otherwise specified, the branches described in the tables of this paper were from ⅝ to ⅞ inch in diameter. Branches as small as ¼ inch in diameter appear to be worse injured. Branches more than an inch in diameter show less streaking than the smaller ones, though the lenticels absorb the arsenicals almost as freely. The thickness of the bark seems to be a factor here. Except for the fact that the branches are eventually killed by girdling under the bandage through anastomosing lenticel spots, the injury does not extend as far from the bandages as in the smaller branches.

Twelve other branches varying in size from ¼ inch to 2½ inches were wounded by cutting off a small twig or by cutting a small notch to the cambium. Each was then treated with 10 gm. of Baker's calcium arsenite. The injury to these limbs was very severe. All less than 1 inch in diameter were killed above the bandage and there were long streaks below. Those more than 1 inch across (except one) were badly injured above the bandage but not entirely killed, and the streaks below were much shorter than on the smaller branches. The one exception just mentioned, which was 1⅛ inches in diameter, was the worst injured of any. It was killed above the bandage and a wide dead streak ran down 4 feet to the trunk and out a large side branch 6 feet to its tip.

It would seem from this that, when branches have been wounded so as to expose both phloem and xylem ducts, there is no consistent difference

in amount of injury between the smaller branches and the larger until a diameter of 1 inch is reached. The portion above the point of treatment is more often entirely killed in the smaller branches than in the larger, but this is because there is less tissue to kill above the point of treatment. The largest two in this series showed somewhat less injury than the rest, but one much larger than the average was the worst injured of the 12.

It is, of course, possible that had a milder chemical been applied than that used in this experiment greater differences due to size of branch would have developed; but with several opportunities during the course of the investigation to make isolated observations along this line we saw no reason to believe that size of branch is an important factor in determining the extent of injury, except that lenticel injury through a thick bark extended less rapidly than through a thin bark. This statement applies only to limbs with a smooth bark.

RELATIVE INJURY BY DIFFERENT ARSENICAL COMPOUNDS

That equal amounts of different chemical compounds injure plant tissues in different degrees is to be expected. In the first place, many of these compounds are readily soluble in water, and others are only slightly so and are often called "insoluble." All the arsenical insecticides used for spraying plants are of the latter character. As the solubles are more readily absorbed than the so-called insolubles when abundant moisture is present, it seems best to compare the solubles with each other and the insolubles with each other. In making such comparisons it would be possible either to use equal weights of the different chemicals or such quantities as to give equal amounts of arsenic. Either basis would be logical for its own purposes, and the former—that is, equal weights of the chemicals—was chosen for this investigation.

Considering, first, the soluble arsenicals, we have compared them on both wounded and unwounded limbs. These chemicals were dissolved in water in proportions between 1 to 1,000 and 1 to 1,000,000 and the bandages were saturated with the solution, evaporation being practically prevented by wrapping with a dental rubber dam.

Twelve unwounded branches from ½ to ¾ inch in diameter were treated with soluble compounds as follows: Two each with arsenic acid, ammonium arsenate, potassium arsenate, potassium arsenite, sodium arsenate, and sodium arsenite in the strengths of 1 to 1,000 and 1 to 5,000 by weight. Arsenic acid in each strength caused a small lenticel spot. Potassium arsenate, 1 to 1,000, caused a few small lenticel spots. Sodium arsenite 1 to 1,000 caused a few small lenticel spots and a spot ⅜ inch in diameter about a latent bud. The others caused no injury.

Thirty-six other branches received two wounds each, either by cutting off twigs or by cutting a small notch through the bark to the cambium.

The same compounds were used as on the unwounded branches just described. The strengths varied between 1 to 10,000 and 1 to 1,000,000. In a strength of 1 to 1,000,000 none of the chemicals caused injury. In a strength of 1 to 100,000 there was no injury other than a slight discoloration of the margin of the wounds, except in the case of potassium arsenate, which in one of the duplicate treatments killed the bark for ⅛ to ⅝ inch around the wound. In a strength of 1 to 50,000 practically no injury was caused by potassium arsenate, potassium arsenite, or sodium arsenate. The bark was killed for $\frac{1}{16}$ to ⅛ inch about the wound by arsenic acid and for ⅛ to ¼ inch by sodium arsenite. Ammonium arsenate caused a spot ½ by 1 inch about the wound. When used in a strength of 1 to 10,000, there was some injury from every chemical, and in most cases definite streaks ran up and down from the wounds. The average length of these streaks from the top of the one running up from the wound to the bottom of the one running down was as follows: Arsenic acid, 16 inches; ammonium arsenate, 2 inches; potassium arsenate, 4 inches; potassium arsenite, ¾ inch; sodium arsenate, ¾ inch; sodium arsenite, 1¼ inches. As these figures are averages for four wounds in each case, they probably give a fair idea of the toxicity of the soluble arsenical compounds.

It may be seen that when the limbs are not wounded they will withstand much stronger solutions than if the bark is ruptured. A strength of 1 to 10,000 materially injured wounded limbs; but 10 times that strength, or 1 to 1,000, caused very little injury to unwounded limbs, and in most cases none at all.

Turning now to a consideration of the relative injuriousness of the so-called insoluble arsenicals, we find much wider differences than in the solubles. Here we have to deal with two factors: The solubility of the chemicals under the conditions of the experiments and the toxicity of the part in solution.

To get a comparison of the effects of different insoluble arsenicals on unwounded branches, the following were applied in 60-gm. quantities:

Arsenic trioxid, Baker.
Arsenic trioxid, Merck.
Arsenic trisulphid, Baker.
Arsenic trisulphid, Merck.
Calcium arsenite, Baker.
Calcium arsenite, Merck.
Copper aceto arsenite, Ansbacher.
Copper aceto arsenite, Baker.
Lead arsenate, diplumbic, Baker.
Lead arsenate, diplumbic, Swift.
Lead arsenate, triplumbic, Baker.
Lead arsenate, triplumbic, Grasselli.
Lead arsenate, technical, Merck.
Zinc arsenite, ortho, Baker.
Zinc arsenite, ortho, California Spray Co.

Two unwounded branches were treated with each chemical. The severest injury was caused by arsenic trioxid, arsenic trisulphid, calcium arsenite, and Baker's triplumbic lead arsenate. These all produced streaks extending up and down from the bandages for several

inches. Under the bandage the branch was girdled by at least one of the duplicate treatments. Zinc arsenite (California Spray Co.) caused no injury whatsoever. The amount of injury caused by the other chemicals was intermediate, consisting of lenticel spots that were anastomosing more or less.

Twelve other unwounded branches were treated in duplicate with the following chemicals, 10 gm. each:

Calcium arsenite, Baker.	Zinc arsenite, ortho, Baker.
Iron arsenate, Merck.	Zinc arsenite, pyro, Baker.
Copper aceto arsenite, Baker.	Zinc arsenite, meta, Baker.

In this series calcium arsenite entirely killed the branch above the bandage. Streaks ran down from the bandages 16 and 20 inches, respectively. Copper aceto arsenite and the three zinc arsenites caused only lenticel spots, which in some cases were anastomosing. Iron arsenate caused no injury.

It is to be noted that in this last series 10-gm. quantities of calcium arsenite and of copper aceto arsenite caused more injury than did 60 gm. in the series just described. The 60-gm. series was begun on July 30, 1911, and the 10-gm. series on June 17, 1912, both in midsummer.

Had the injury in these two series been approximately equal, it would not have been especially remarkable, as we have found that, as a rule, the injury from these slightly soluble arsenicals is by no means proportional to the amounts used. Erratic results like these have made us very careful about drawing conclusions concerning the exact amounts required to produce injury, and have prompted us to make comparisons only between treatments given on the same date under conditions as nearly alike as possible.

We may at least conclude that even unwounded limbs may be severely injured or ultimately killed as a result of girdling by some of the chemicals having only slight solubility. It appears, however, that tests on wounded limbs, where the exposure of living tissues to the chemicals is assured, are somewhat more suitable for comparing the toxicity of the chemicals than those on unwounded limbs where the corky bark limits their free access.

Tables IV and V record the results of work specifically intended to determine the relative injuriousness of the more common insoluble arsenical compounds when applied to branches that are wounded. A close study of these tables shows some points of interest. One of the most striking facts is that similar tests, made under conditions as nearly alike as possible, do not always yield the same result. Briefly stated, duplicate tests do not always check with each other, though in most cases they do so fairly well. In other words, there are factors that we can not control, and do not even know, that sometimes influence to a marked degree the

extent of injury. This introduces an experimental error that must not be overlooked. Iron arsenate and one brand of zinc arsenite (California Spray Co.) have been conspicuous in all the tests by the small amount of injury which they produce. Other brands of zinc arsenite have proved more injurious. It is obviously unsafe to make general statements about "ortho zinc arsenite" without naming the brand. Indeed, we have no assurance that different lots of any brand will always be harmless. The same may be said of the lead arsenates. Even those manufactured as pure diplumbic or triplumbic show some differences. Two arsenicals have quite regularly given severe injury: Calcium arsenite and arsenic trioxid. Arsenic trisulphid has usually given less injury than these and copper aceto arsenite still less, though more, as a rule, than the ortho zinc arsenites and most lead arsenates.

TABLE IV.—*Results of wounding and treating branches of Hibernal apple on June 23, 1911, with different arsenicals to show their relative injuriousness. Those with even numbers were covered with a dental rubber dam and those with odd numbers were not* (*see p. 286*)

No.	Chemical.	Extent of injury.			
		60 gm. of chemical.		10 gm. of chemical.	
		Above bandage.	Below bandage.	Above bandage.	Below bandage.
			Inches.		*Inches.*
1	Arsenic trioxid, Baker	Entire branch.	44	Entire branch.	[a] 31+
2	do	do	27	do	14
3	Arsenic trioxid, Merck	do	29	do	29
4	do	do	41	do	25
5	Arsenic trisulphid, Baker	do	29	do	16
6	do	do	33	do	13
7	Arsenic trisulphid, Merck	11 inches	4	do	34
8	do	Entire branch.	18	do	8
9	Calcium arsenite, Baker	do	22	do	[b] 9+
10	do	do	39	do	18
11	Calcium arsenite, Merck	do	[b] 14+	do	[a] 10+
12	do	do	[b] 16+	do	13
13	Lead arsenate, diplumbic, Baker	do	40	do	15
14	do	do	29	do	6
15	Lead arsenate, diplumbic, Swift	Streak to tip.	2	1 inch	1
16	do	Entire branch.	5	18 inches	1
17	Lead arsenate, triplumbic, Baker	do	2	36 inches	2
18	do	do	5	Entire branch.	6
19	Lead arsenate, triplumbic, Grasselli	do	20	do	5
20	do	Streak to tip.	3	3 inches	3
21	Lead arsenate, technical, Merck	Entire branch.	7	Entire branch.	3
22	do	do	11	Streak to tip.	5
23	Copper aceto arsenite, Baker	do	18	12 inches	10
24	do	do	12	Entire branch.	2
25	Copper aceto arsenite, Ansbacher	do	54	do	9
26	do	do	12	Streak to tip.	3
27	Zinc arsenite, ortho, California Spray Co	None	None.	None	None.
28	do	do	do	do	Do.
29	Zinc arsenite, mixture, Baker	Entire branch.	18	Entire branch.	10
30	do	do	11	Streak to tip.	5
31	Zinc arsenite, mixture, Merck	do	13	8 inches	1
32	do	do	19	Entire branch.	1

[a] No. 1 (10 gm.) and 11 (10 gm.) grew from the same main limb and the streaks ran together on it.

[b] Nos. 9 (10 gm.), 11 (10 gm. and 60 gm.), and 12 (60 gm.) grew from the same main limb, and the streaks from all three bandages ran together upon it, making it impossible to say how much of the common streak was caused by any one of them.

TABLE V.—*Results of wounding and treating branches of apple on June 17, 1912, with different arsenicals, 10 gm. each, to show their relative injuriousness*

No.	Chemical.	Extent of injury.	
		Above bandage.	Below bandage.
			Inches.
1	Calcium arsenite, Baker	Entire branch.	19+
2	....do	....do	20
3	Iron arsenate, Merck	½ inch	½
4	....do	6 inches	2
5	Copper aceto arsenite, Baker	36 inches	12
6	....do	Entire branch.	5
7	Zinc arsenite, ortho, Baker	9 inches	8
8	....do	30 inches	2
9	Zinc arsenite, pyro, Baker	10 inches	2
10	....do	Streak to tip.	2
11	Zinc arsenite, meta, Baker	Entire branch.	12
12	....do	....do	32

[a] Nineteen inches below the bandage this branch joined a main limb bearing a dead streak from another bandage, and the two ran together.

It is interesting to compare in Table IV the relative amounts of injury from 60 gm. and 10 gm. of the insoluble arsenicals under identical conditions. While there is somewhat greater injury, as a rule, from the larger amount, it is not at all proportional to the weights used.

Table IV also shows that there was no consistent difference between the injuries under bandages covered by a rubber dam and those not so covered, but kept moist by frequent watering. On the whole, they compare about as well as the duplicates in the other tables.

In general, the injury is much worse on wounded limbs, and nearly all the so-called insoluble arsenicals are capable of doing much damage to the tree if they gain access to the living tissues.

EFFECTS OF WASHING THE ARSENICALS

The question has arisen with much force whether the injuries produced in the course of this investigation were due to a portion of the arsenical going into solution and exerting a toxic action or to an impurity of a more or less soluble nature. This is indeed an important matter, for an assumption here without knowledge of the facts may lead one into a serious and fundamental error. In our efforts to settle this question we have, in the first place, obtained most of our chemicals from reliable companies. In some cases we have had special lots manufactured for our use, with the distinct understanding that regardless of cost they must be free from all soluble arsenical impurities. All of the arsenicals received from the Baker Chemical Co. (see p. 287) were of this character. To test further the possibility of soluble impurities we washed one lot of each of the more important chemicals with many changes of distilled water. These were then tested in comparison with others from the same containers but unwashed.

The results show that the washing may have slightly decreased the injury in some cases. This was not uniformly true, for with arsenic trioxid and ortho zinc arsenite the washed chemical caused fully as much injury as the unwashed, and with copper aceto arsenite and triplumbic lead arsenate the results were nearly alike. In fact diplumbic lead arsenate was the only chemical that showed a striking improvement as a result of washing. This is probably due, as suggested by Dr. Headden,[1] to a change in the composition of the lead arsenate from the diplumbic to the triplumbic as a result of washing. A close comparison of the results is fruitless, owing in part to the rather large experimental error of the methods here used. The essential fact is that both washed and unwashed chemicals of the purest brands obtainable do cause severe injury.

EFFECTS OF ADDING LIME TO THE ARSENICALS

It has frequently been recommended that lime be added to arsenical insecticides, especially copper aceto arsenite, to lessen the possibility of injury when sprayed on foliage. To determine whether this would materially lessen the injury under the conditions of these experiments—namely, when the arsenicals are applied to the wounded bark—1 gm. of partly air-slaked lime [2] was mixed with the arsenical contained within each bandage. The following arsenicals, 10 gm. each, were used, two with lime and two without:

Calcium arsenite, Baker.
Copper aceto arsenite, Baker.
Lead arsenate, diplumbic, Baker.
Lead arsenate, diplumbic, Grasselli.
Lead arsenate, triplumbic, Baker.
Lead arsenate, triplumbic, Grasselli.
Zinc arsenite, ortho, Baker.
Zinc arsenite, "Ortho 40," California Spray Co.
Lime only.

A comparison of the injury with and without lime shows little evidence that lime lessens the injury from any of the chemicals tested. It appeared at first glance to lessen the injury from Grasselli's triplumbic lead arsenate and Baker's ortho zinc arsenite, but as it failed to do so with Baker's triplumbic lead arsenate and "Ortho 40" zinc arsenite, we are led to believe that this apparently beneficial effect is largely a matter of coincidence. We are strengthened in this belief by the fact that several duplicate tests in this series differ so much from each other. The lime alone caused no injury.

DIFFERENCES IN VARIETIES OF APPLE, PEAR, AND PLUM

Field observations have led to the belief among horticulturists that there is a difference in varieties of apple with reference to the resistance of their foliage to injury by arsenical spray mixtures. The Ben Davis

[1] This suggestion was offered verbally by Dr. Headden in 1910.

[2] Quicklime was allowed to air-slake until it had taken a powdered form, but was still strongly alkaline to litmus paper.

is reported to be easily injured, and the Gano shares somewhat this reputation, not so much apparently from actual observation as from inference because of the close relationship of the two varieties. Headden (5, 7) refers to this special susceptibility of the Ben Davis and the Gano and notes that under some conditions at least these two varieties are most frequently victims of crown injury. So far as the spray injury is concerned, this special weakness of the Ben Davis and the Gano may be due either to poorer protection of the leaf cuticle (or possibly to some difference in the stomata) or to a greater susceptibility of the living interior portion. Likewise, if the injury described by Headden is caused by arsenic which has accumulated about the crown, the more frequent injury which he observed in these varieties may have been due to a structural difference in the protective outer bark or to a difference in the resistance of the inner tissues.

More than 100 tests were made on wounded and unwounded branches for direct comparisons, under practically identical conditions, of the relative susceptibility of varieties. The following were included in these tests: Alexander, Ben Davis, Gano, Hibernal, Okabena, Wealthy, and Yellow Transparent apples; Hyslop and Transcendent crab apples; Flemish Beauty pear, and De Soto and Moldarka plums.

In addition to the tests planned for this specific purpose, many others made for different purposes gave additional opportunity for comparisons of varietal susceptibility. A fairly representative series is shown in Table VI.

TABLE VI.—*Results of wounding (twig cut off or notch cut to cambium) and treating different varieties of apple and plum on June 14, 1915, with 10 gm. of Baker's copper aceto arsenite, to compare susceptibility of varieties*

No.	Diameter of treated branch.	Variety.	Extent and character of injury.
	Inch.		
1	5/16	Ben Davis apple.....	Dead above bandage except for narrow strip that runs up 14 inches. Streak runs down 2 inches. Several lenticel spots.
2	5/16	.....do.....	Streak nearly around branch runs up 5 feet to tip. Narrow streak runs down 3 inches. Nearly girdled under bandage.
3	5/16	.....do.....	Dead above bandage except for narrow strip that runs up 1 foot. Streak half around branch runs down 10 inches.
4	5/16	Gano apple.....	Dead above bandage except for narrow strip that runs up 10 inches. Streak runs down 6 inches.
5	5/16	.....do.....	Streak half around branch runs up 4 feet to tip and down 6 inches. Large spot at latent bud.
6	3/8	.....do.....	Streak half around branch runs up 3 feet to tip and down 10 inches to main limb and on down this 6 inches.
7	5/16	Hibernal apple.....	Dead above bandage except for narrow strip that runs up 2 feet. Streak runs down 2 inches.
8	5/16	.....do.....	Dead above bandage. Streak half around runs down 6 inches to main limb.
9	5/16	.....do.....	Wide streak runs up 22 inches. No injury below bandage. Several lenticel spots.
10	5/16	Okabena apple.....	Streak two-thirds around branch runs up 3 feet to tip. Wide streak runs down 6 inches. Three lenticel spots.
11	5/16	.....do.....	Dead above bandage except for narrow strip that runs up 16 inches. Wide streak runs down 2 feet to main limb. Several lenticel spots.
12	5/16	.....do.....	Streak two-thirds around branch runs up 4 feet to tip. No injury below bandage. Several lenticel spots.
13	5/16	Oldenburg apple.....	Streak half around limb runs up 9 inches to side branch and out this 14 inches. Wide streak runs down 7 inches. Several lenticel spots.
14	5/16	.....do.....	Dead above bandage except for narrow strip that runs up 15 inches. Streak half around runs down 7 inches.

TABLE VI.—*Results of wounding (twig cut off or notch cut to cambium) and treating different varieties of apple and plum on June 14, 1915, with 10 gm. of Baker's copper aceto arsenite, to compare susceptibility of varieties*—Continued

No.	Diameter of treated branch.	Variety.	Extent and character of injury.
15	5/8	Oldenburg apple.....	Dead above bandage except for narrow strip that runs up 5 inches. Two streaks run down 5 inches.
16	5/8	Wealthy apple.....	Streak half around branch runs up 3 feet to tip and down 4 inches. Several lenticel spots.
17	5/8	.....do.....	Do.
18	5/8	.....do.....	Streak half around branch runs up 3 1/2 feet to tip. Narrow streak runs down 3 inches. Several lenticel spots.
19	5/8	Yellow Transparent apple.	Streak half around branch runs up 5 feet to tip. Narrow streak runs down 5 inches. One lenticel spot.
20	5/8	.....do.....	Streak half around branch runs up 2 feet. Wide streak runs down 7 inches. Several lenticel spots.
21	3/4	.....do.....	Wide streak runs up 2 1/2 feet. Narrow streak runs down 5 inches. Several lenticel spots.
22	1/2	Hyslop crab.....	Dead above bandage except for narrow strip that runs up 6 inches. Wide streak runs down 8 inches.
23	5/8	.....do.....	Streak two-thirds around branch runs up 2 1/2 feet to tip. Wide streak runs down 5 inches. Many lenticel spots.
24	5/8	.....do.....	Streak half around branch runs up 18 inches and down 3 feet. Several lenticel spots.
25	3/4	Transcendent crab...	Dead above bandage. Wide streak runs down 8 inches.
26	1/2	.....do.....	Streak three-fourths around branch runs up 4 feet to tip. Streak runs down 3 inches.
27	1/2	.....do.....	Streak half around branch runs up 5 feet to tip. No injury below bandage.
28	1/2	De Soto plum.....	Streak half around branch runs up 3 feet to tip. Streak runs down 8 inches.
29	5/8	.....do.....	Streak half around branch runs up 2 1/2 feet to tip and down 2 inches.
30	1/2	.....do.....	Streak half around branch runs up 3 1/2 feet to tip. Wide streak runs down 5 inches to main limb and on down this 1 foot.

Throughout our work we find little evidence of any important difference in the susceptibility of varieties of apple to arsenical injury when the chemical is admitted through wounds. If conclusions were drawn from this table alone, it might be inferred that the Wealthy is somewhat more resistant than the others; but this is not borne out by other tests. It may be said further of the tests reported in this table that the triplicates check with each other fully as well as the majority made in this investigation. The few tests made of pear and plum lead to the belief that they are not very different from apple under these conditions—namely, when the arsenical is applied to wounded branches 1/2 to 3/4 inch in diameter.

In the tests on unwounded branches to compare the susceptibility of varieties, 10 gm. of copper aceto arsenite were used in most cases. This produced lenticel spots but no streaking on every treated branch of every variety of apple tested. The number and size of these spots seemed to vary about as much between the duplicates or triplicates on the same variety as between varieties, and we got no evidence of special susceptibility or resistance in any variety of apple. In the Flemish Beauty pear there was a much greater tendency for the lenticel spots to enlarge and anastomose, and in the Moldarka plum there was a discoloration of all the outer bark which rarely reached the cambium. Possibly, if the bandages had been kept on for a very long time, a gradual extension of the injury might have taken place in which some varietal difference would have been apparent.

From these repeated experiments, therefore, it seems safe to conclude that when arsenicals are applied by this method to either wounded or unwounded limbs they injure the different varieties of apple about equally. Certainly neither the Ben Davis nor the Gano is especially susceptible under these conditions. However, as the governing factor seems to be the outer protective covering, and as this covering is very different on the smooth-barked limbs and on the rough-barked crowns extensive tests on the latter might yield different results. No Ben Davis or Gano trees were available for crown treatment, however, and many might be required to make conclusions free from experimental error. Without these two varieties such a test would miss the point of greatest interest.

EFFECTS OF SEASON UPON INJURY

As this investigation was carried on during several months of spring, summer, and fall, it is of some importance to know whether the season or stage of growth influences the extent or character of injury. To determine this, a considerable number of tests was made during every month from March to October, inclusive. From the specific treatments made for this purpose and others that have contributed to our information on the relation of season to injury, we conclude that when the trees are frozen the injury is very restricted, being confined wholly to the exposed tissues of the wounds. When the trees are more or less active, even before the leaves come out in the spring and after they drop in the fall, the injury will readily extend a considerable distance from the point of treatment. Throughout this active period the extent of injury from a given treatment is about the same, but it runs its course a little more rapidly just after the leaves come out and more slowly about the time they are dropping in the fall.

INJURY THROUGH THE ROUGH BARK OF LIMBS, TRUNKS, AND CROWNS

Casual observation might lead one to believe that the heavy, rough bark on the trunks of trees is a better protection against all kinds of harmful agencies than the thin, smooth bark of the small branches. That it does offer a greater protection against mechanical injuries is undisputed, and it is also true that the thicker, projecting portions of the rough bark are highly protective against the absorption of arsenical compounds when added to the unwounded surface. Between these thick ridges however, are the furrows in which the corky layer is more or less broken. As the tree expands in diameter new rifts are formed or the old ones are reopened. Microscopic study confirms what close macroscopic observation suggests, namely, that the rough bark has vulnerable points of a character not found in the periderm of the branches above.

It has often been observed that when a tree is thoroughly drenched with a spray mixture a considerable quantity of this runs down the trunk

to the ground. Some lodges in cracks and other irregularities of the bark on the trunk; but this is not likely to cause injury, as it soon dries out. Around the crown, however, it accumulates to a much greater extent and is often kept moist by the soil for long periods of time. As the crown of the tree is covered by the rough or furrowed bark which theoretically offers only partial protection, it is highly important to determine its vulnerability by actual test.

INJURY TO LARGE LIMBS WITH ROUGH BARK

A few large limbs were found having longitudinal cracks in the bark of the kind just described. These limbs were treated with copper aceto arsenite or calcium arsenite under bandages such as were used for most of this investigation. Care was used to make no artificial wounds. The results are shown in Table VII.

TABLE VII.—*Results of treating large unwounded limbs of apple showing the first cracks of rough bark formation, on July 13, 1912, to show injury through rough bark*

No.	Chemical (50 gm.).	Diameter of treated limb.	Natural cracks in bark, apparently recent.	Extent and character of injury.
		Inches.		
1	Copper aceto arsenite, Baker.	3¼	One short, deep, longitudinal crack 5 inches long.	Limb completely girdled under bandage and for about 2 inches above.
2	.....do.....	3⅜	Irregular longitudinal crack about 3 inches long.	Limb girdled under bandage and for about 3 inches above and below.
3	.....do.....	2¾	Deep longitudinal crack 6 inches long.	Limb girdled under bandage and about 3 inches above and below. Streak runs up 10 inches.
4	Calcium arsenite, Baker.	2¾	Longitudinal crack 7 inches long. Lower portion recently formed.	Limb girdled under bandage and about 7 inches above and 3 inches below.
5	.....do.....	4	Six longitudinal cracks on under side of limb. Not all recent.	Limb girdled under bandage. Wide streak runs up 2½ feet and down to ground 2½ feet.
6	.....do.....	1¾	Irregular cracking on one side...	Limb girdled under bandage. Streak runs up 2 feet and down 6 inches.

It will be seen from this table that in every case the limb was severely injured. The absorption was rather slow and there was little tendency to form the dead, sunken streaks up and down the limbs so characteristic of injury through wounds. In this respect there was a close similarity to the crown injury under controversy in Colorado and elsewhere. Lenticel injury was out of the question in these cases, as the lenticels had become nonfunctional and indeed had practically disappeared from the larger limbs.

INJURY TO TRUNKS

A few trunks also were treated early in the course of this investigation. The method here was to apply the chemicals in funnels about the trunks as described on page 286.

That the bark on the trunk fails to protect the tree from arsenical injury is shown by Table VIII. The absorption was evidently slow,

judging from the absence of symptoms in the foliage for several weeks after the trunks were treated. Owing to the dark color and firm texture, the heavy bark does not show externally the injury that is taking place until long after the tissues are dead; and it seemed undesirable to disturb the experiments by cutting into the bark until there was other evidence that injury had taken place.

TABLE VIII.—*Results of treating the unwounded trunks of apple trees with different chemicals applied on July 12, 1912, to show injury through rough bark*

No.	Chemical.	Injury 2½ months after treatment.[a]	Injury 8½ months after treatment.
1	Sodium arsenite, Merck, 10 gm.	Bark dead for 8 to 12 inches above funnel. Nearly half the leaves killed. Bark and wood of branches not discolored.	Tree dead throughout. Bark dark and friable below and under funnels, especially around the crowns where covered by earth.
2	.....do.....	Bark above funnel nearly all sound. Streak runs up 2 feet. About half the leaves killed and many of the remainder spotted.	Do.
3	Arsenic trioxid, Baker, 500 gm.	Two streaks run up trunk about 1 foot. No leaves killed or spotted. About half the fruit softening and streaked throughout with brown. No untreated trees have fruit in this condition.	Trunk girdled under funnel. Streak runs up 6 inches. Bark dead below funnel to the roots. Rather dark colored and friable.
4	.....do.....	No dead bark above funnel. Otherwise like No. 3.	Trunk girdled under funnel. Streak runs up 2 feet. Otherwise like No. 3.
5	Copper aceto arsenite, Baker, 500 gm.	No dead bark above funnel. Fruit softened and streaked like No. 3.	Trunk girdled under funnel and irregular patch of dead bark extends several inches above. Bark below funnel killed on one side of trunk to roots. Below ground it is dark reddish brown and friable.
6	.....do.....	No visible injury to bark, leaves, or fruit.	Trunk girdled under funnel. Wide streak on one side extends from funnel nearly to ground.
7	Lead arsenate, triplumbic, Baker, 500 gm.	Bark sound above bandage. Fruit softened and discolored like No. 3.	Funnels left for later notes (see first paragraph following this table.
8	.....do.....	No visible injury to bark, leaves, or fruit.	Do.
9	Water only (control)..	No visible injury.....	No injury of any kind.

[a] When these first notes were taken, the funnels were not removed and the injury below the funnels could not be traced externally. When the last notes were taken the funnels were removed and the trees cut up.

It will be noted from the table that No. 7 and 8, the two trees treated with lead arsenate, were not disturbed at the time the notes were taken, 8½ months after treatment. It was desired to ascertain whether or not the injury would continue, these two being the only trees not girdled and therefore the only ones we could hope would survive. One of these trees, No. 7, was removed from the orchard through a misunderstanding before final notes were taken. The other, No. 8, showed no injury externally on October 28, 1914, 2¼ years after treatment. On removing the funnel and cutting into the bark on that date, however, a dead spot was found under the bandage from which a streak ran down to the roots.

It is worthy of note that in these cases where the absorption was slow the visible injury was not always continuous as where the absorption was rapid through a wound. Most of the bark and sapwood between the funnel and the discolored leaves in No. 1 and 2, 2½ months after treatment, was normal in appearance, and in No. 3, 4, and 7 most of the bark and sapwood was not discolored 8½ months after treatment, although the fruit had shown injury the previous September.

INJURY TO CROWNS

In treating the crowns of trees it was deemed advisable to place the chemicals very much as they accumulate as a result of spray mixtures running down the trunks. To accomplish this, the earth was removed around the crowns to a depth of a few inches, care being taken not to injure the bark in the slightest degree, except on certain trees that were purposely wounded. The arsenicals were then distributed in these shallow trenches against the bark and the earth replaced. Except in applying the chemicals, no water was added during the course of the experiments, the natural moisture of the soil being depended upon to keep some arsenic in solution at least a portion of the time. Fresh applications were given in three successive years, the last one being twice the strength of the first two.

The experiments recorded in Table IX show that even small quantities of arsenic trisulphid or copper aceto arsenite are capable of causing injury to the unwounded crowns. This suggests that in time the injury might even girdle the trees. That calcium arsenite would have caused a more serious injury in the same length of time seems highly probable, judging from a comparison with the other chemicals when applied to branches. We must not, however, overlook the possibility that the soil solution about the crowns influenced the relative injuriousness of the chemicals.

TABLE IX.—*Results of treating the crowns of apple trees three successive years with different chemicals, to determine character of injury*

No.	Treatment.	Diameter of trunk.	Wounds at crown.	Injury, Mar. 25, 1913.
		Inches.		
1	Arsenic trioxid, Merck; 7 gm. July 19, 1910, and Aug. 22, 1911; 14 gm. Aug. 8, 1912.	4½	Unwounded.....	Uninjured.
2	.....do.....	4¼	Two strips of bark ¾ by 1¼ inches removed just below ground.	Narrow streak runs up 3 feet. Roots uninjured.
3	Arsenic trisulphid, Merck; 15 gm. July 19, 1910, and Aug. 22, 1911; 30 gm. Aug. 8, 1912.	4¾	Unwounded.....	Several dead spots about 1 inch in diameter extend into bark, but none reach the cambium.
4	.....do.....	4¼	Same as No. 2.....	Wide streak runs up 3 feet, but does not extend down onto roots. Several dead spots about 1 inch in diameter extend into bark. The tissue is very dark and friable. Two spots extend through bark into wood.
5	Lead arsenate, technical, Merck; 60 gm. July 19, 1910, and Aug. 22, 1911; 120 gm. Aug. 8, 1912.	2	Unwounded.....	Apparently uninjured Aug. 8, 1912. Removed before final notes were taken.
6	.....do.....	4½	Water sprouts cut from crown.	Do.
7	Copper aceto arsenite, Ansbacher; 15 gm. July 19, 1910, and Aug. 22, 1911; 30 gm. Aug. 8, 1912.	3¼	Unwounded.....	Several small dead spots on crown, but none reached the cambium.
8	.....do.....	3	Water sprouts cut from crown.	Wide dead streak runs up 4 feet. This starts from a large dead patch on the crown. Roots uninjured.
9	Zinc arsenite, ortho, California Spray Co.; 15 gm. July 19, 1910, and Aug. 22, 1911; 30 gm. Aug. 8, 1912.	4¼	Unwounded.....	No injury.
10	.....do.....	4	Same as No. 2.....	Tree broken down by storm.

When trees are wounded at the crown, as by the cutting off of water sprouts, which is a fairly common orchard practice, the danger of arsenical injury is greatly increased, as shown by a comparison of the wounded and unwounded trees in this table.

To determine whether a more rapid injury could be produced by larger applications of more injurious chemicals, the tests were made that are recorded in Table X. A study of this table leaves no doubt that the crown of an apple tree is very poorly protected against the absorption of arsenicals. Three days after treatment nearly every tree showed some symptoms in the foliage and at the end of a week every tree was badly injured. In a few weeks every crown was completely girdled.

TABLE X.—*Results of treating unwounded crowns of apple trees on July 19, 1912, with 500 gm. of Baker's calcium arsenite, to determine the extent and character of injury*

No.	Diameter of trunk.	Injury 1 week after treatment.	Injury 9 weeks after treatment.	Injury 8¼ months after treatment.
	Inches.			
1	3	No injury except the killing of water sprout at base of tree.	Crown girdled. No streaking. Foliage strongly purplish in color.	Bark around crown below ground very dark and friable.
2	3	Streak runs up trunk on north side and out onto several limbs. More than half the leaves, especially on north side, badly injured.	Crown girdled. One wide streak and one narrow one run to top of tree. About one-third the leaves killed and others spotted. The rest have a strongly purplish color. Fruit small and highly colored, except on killed limbs, where it is dead and shriveled.	Bark at crown not so dark as No. 1 and rather tough instead of friable.
3	3⅛	Faint streaks run from trunk onto several limbs. About two-thirds the leaves, especially on southeast side, badly injured.[a]	Crown girdled. Streak three-fourths around trunk runs to top of tree. About three-fourths the leaves killed and others spotted. The rest normal in color.	Bark at crown rather dark and more friable than after 9 weeks.
4	3⅛	Leaves of four spurs on main limb badly injured.	Crown girdled. Wide streak runs to top of tree. About one-fourth the leaves killed or spotted. The rest normal in color.	About like No. 3.
5	6	Faint streaks run from trunk onto limbs. About one-fourth the leaves injured.	Crown girdled. One wide and one narrow streak run to top of tree. About one-eighth the leaves killed or spotted, the rest purplish in color.	Bark at crown very dark and friable.
6	9	Many leaves badly injured.	Girdled at crown and for 18 inches above ground. Several streaks run to top of tree. About one-half the leaves dead and many others spotted, the rest normal in color. Fruit dark and soft at core.	Do.

[a] In removing the soil from crown for treatment a slight wound, not through the bark, was accidentally made on west side.

Of much significance in reference to the interpretation of these results (see p. 314) is the change that takes place under ground. On September 26, nine weeks after treatment, the color of the dead bark below and above ground was about the same—namely, about the same shade of brown as a roasted coffee berry. By the following March the color below the ground was a very much darker brown, especially that of No.

1, 5, and 6, while above the ground it had darkened but slightly. A corresponding change had taken place in the texture of the bark. All the dead bark was very tough and fibrous when first killed, and on all aerial portions it remained so, but on the crowns below the surface of the ground it gradually became so disintegrated, especially on those with the darkest color, that it could easily be picked apart with the fingers. When dry, it was rather brittle; when wet, it was so soft as to be almost pasty in certain portions. It seems probable that this disintegration is largely due to the invasion of saprophytic microorganisms rather than to the direct action of the arsenicals.

Comparing the injury through rough bark with that through the smooth bark of the crown, in both cases unwounded, we find that the smooth bark is vulnerable through lenticels and the rough bark through cracks or fissures caused by growth in the cambium region. Either is sufficient to make possible the girdling of the tree or limb at the point of application. Which permits the more rapid absorption depends upon conditions and is, indeed, of little moment.

INJURY THROUGH ROOTS

As most of the spray mixture applied to a tree eventually reaches the ground under it, the likelihood of injury to the roots or to the whole tree through the roots is a subject of interest.

INJURY FROM BANDAGES

A few roots were treated by the bandage method used largely on the branches. The number so treated, however, was relatively small for two reasons: First, the amount of work required is vastly greater than if branches are used, and, second, it is almost impossible to uncover roots and apply bandages to them without wounding them. Most apple roots of a suitable size are covered with fine rootlets the removal of which makes wounds that readily admit the arsenic. However, enough were found free from all side roots to make it possible to establish the following facts: (1) That the unbroken periderm is quite resistant to the absorption of arsenical compounds, (2) that some of the lenticels will admit it, and (3) that when admitted through a wound the effect is about the same as in a branch of the same size. Indeed, we came to regard the susceptibility of roots ½ inch in diameter or larger as about the same as that of branches of equal size.

INJURY BY ABSORPTION THROUGH THE FINE FEEDING ROOTS

In addition to the possibility of a localized injury of crowns or roots by a heavy accumulation of arsenical insecticides that have run down the trunks, we must consider the likelihood of small amounts mingling

with the soil and being taken up in solution with the soil water normally absorbed by the roots. It is natural to suppose that some is absorbed in this way, and Headden's work convinces one that the arsenic thereby accumulated in the tissues of the tree in sufficient amounts may be readily determined by chemical analysis. Whether or not the trees are materially injured by arsenic absorbed in this way is a debatable question.

To determine whether or not a visible injury can easily be brought about in this way, arsenic trioxid was added to the soil under three trees and sodium arsenite was added to the soil under three others. The chemicals were mixed with water in the proportion of 1 to 1,000 by weight. The sodium arsenite was completely dissolved before being applied. The chemicals were sprinkled on the surface of the ground with an ordinary watering pot. Repeated treatments were given, as shown in Table XI. The first application was made to a circular area of ground 8 feet in diameter, with the tree in the center. The second was made in a ring 2 feet wide just outside the first treatment. Subsequent applications alternated between these two areas of ground.

No crop was grown in the orchard, and the ground was harrowed at frequent intervals but not plowed. The trees were irrigated by shallow furrows running about 4 feet from them. The precipitation during the experiment was as follows:

1911.

	Inches.		Inches.
August	1.52	November	1.00
September	1.73	December	.65
October	2.15		

1912.

January	0.92	July	1.91
February	.39	August	1.63
March	1.86	September	1.66
April	3.59	October	3.23
May	2.66	November	.37
June	2.69	December	.74

1913.

January	0.78	March	0.44
February	.47	April	1.65

No external symptoms of injury appeared in the aerial portion of any of the trees during the entire course of the experiment. The roots were not examined until the end of the experiment, for obvious reasons. Such injuries as were present in the roots at that time are recorded in Table XI. They were all strictly local, with no suggestion of a general or systemic poisoning.

TABLE XI.—*Results of treating the soil underneath apple trees with arsenical compounds to determine injury from absorption by roots*

No.	Chemical.	Amounts and dates of application.	Injury.
1	Arsenic trioxid....	1911: Aug. 22 and Sept. 4, 20 gm. 1912: June 18 and 20, July 2, 9, 16, and 30, 20 gm.; Sept. 4, 40 gm.; Sept. 25 and Oct. 18, 80 gm. Total, 360 gm.	Two roots have outer bark slightly injured. A few rootlets killed. Leaves and branches uninjured.
2	.....do.....	1911: Aug. 22 and Sept. 4, 20 gm. 1912: June 18 and 25, July 2, 9, 16, 30, and Sept. 4, 20 gm.; Sept. 25, 40 gm. Total, 220 gm.	Leaves and branches uninjured. One root has outer bark injured. A few rootlets killed at the tips.
3	.....do.....	.....do.....	Leaves and branches uninjured. A few rootlets killed.
4	Sodium arsenite...	1911: Aug. 22 and Sept. 4, 25 gm. 1912: June 18 and 25, July 2, 9, 16, and 30, 25 gm.; Sept. 4, 50 gm.; Sept. 25 and Oct. 18, 100 gm. Total, 450 gm.	Leaves and branches uninjured. Three roots have outer bark covered in places with anastomosing spots that do not extend to the cambium. Many dead rootlets.
5	.....do.....	1911: Aug. 22 and Sept. 4, 25 gm. 1912: June 18 and 25, July 2, 9, 16, 30, and Sept. 4, 25 gm.; Sept. 25, 50 gm. Total, 275 gm.	Leaves and branches uninjured. Bark of roots slightly injured. Many rootlets killed.
6	.....do.....	Same as No. 5, except that on Sept. 25 only 25 gm. were used. Total, 250 gm.	Leaves and branches uninjured. No injury to large roots and very little to rootlets.

In the spring of 1913 it became necessary to remove the entire orchard block containing these trees, otherwise the applications to the soil would have been continued to date or until the trees had been severely injured.

One conclusion is obvious, that considerable quantities of arsenic may be applied to an orchard soil relatively free from alkali without immediate harmful results. It should, perhaps, be added that the orchard in which this work was carried on is relatively free from alkali and that soils rich in certain alkali salts might keep more of the arsenical in solution.

Too much significance must not be placed on the fact that a soluble compound (sodium arsenite) was applied to some of the trees, as we have no assurance that a large proportion was not rendered insoluble by the action of the soil.

SYSTEMIC POISONING

It is a noteworthy fact that the injuries described thus far in this paper were for the most part local. In fact, all were purely local except those that were a result of girdling limbs or trunks.

It is a pertinent question whether or not trees can be made to suffer a general or systemic poisoning from arsenic, and still more pertinent whether or not such a poisoning ever occurs as a result of orchard spraying as now practiced by fruit growers. Headden has described certain symptoms which he suspects are due to systemic poisoning, but he does not regard the evidence as conclusive.

Our own work on this subject has been meager and does not warrant very far reaching conclusions. It has been confined to two lines: (1) The treatment of the soil recorded in Table XI, and (2) observations on trees that have been treated with bandages on nearly every branch so

that almost the entire top of the tree is killed. In the former case, there were no general symptoms of injury during the course of the experiment. In the latter case, with nearly all the smaller branches killed and streaks running down the larger limbs and trunks, the few remaining branches grew vigorously and the foliage appeared perfectly healthy and normal. As a result of so many branches being killed, many buds pushed out below, making succulent water sprouts. It is well recognized that most of the arsenic absorbed through wounds in the bark may be deposited in the wood and bark that are killed and not carried extensively through the tree. That some is carried into the unkilled portions is shown by analyses made by the chemistry department of this Station. The work of Headden has established beyond question that considerable amounts may be taken up from the soil before the tissues are actually killed. All things considered, we think it doubtful if general systemic poisoning has occurred to any considerable extent as a result of orchard spraying, though we have no reason to believe that it can not be produced experimentally.

SIGNIFICANCE OF RESULTS

The results of the foregoing experiments are of interest chiefly in relation to the pioneer work done by Headden, of the Colorado Agricultural Experiment Station, on the crown injury to fruit trees in Colorado and elsewhere and the relation of spraying for codling moth to this injury. Headden's work was reviewed in the opening pages of this paper, and certain points of strength and of weakness in his evidence were pointed out.

In this contribution we have shown that arsenical compounds used for spraying can penetrate the corky layer of the rough bark and cause a corrosion and killing of the inner bark and produce symptoms similar to those described by Headden. The symptoms we have produced should be closely compared with those found in Colorado. They are alike in several respects: (1) The bark is killed and discolored in a localized area at and extending out from the point in contact with the arsenic; (2) the wood underneath the dead bark is discolored in an irregular region growing smaller toward the center of the tree (if there is no blackheart from winter injury) and up and down from the point of injury; (3) the dead bark, if in contact with moist soil, becomes darker, more or less disintegrated, and friable; (4) the fruit on trees with corroded crowns may become small and highly colored; (5) in early fall the foliage may take on a distinctly purplish color. There are two points of difference: (1) There is a marked tendency for longitudinal dead streaks to be formed as a result of artificial treatment; (2) we have never produced corroded crowns that were quite so dark in color or quite so friable in texture as those on some of the injured trees in Colorado.

These comparisons are worthy of further analysis. There is, on the whole, a rather striking similarity if we restrict our comparisons to injuries on the thick, rough-barked crowns, trunks, and limbs, where the treatment was not too severe and the injury was correspondingly slow. The streaking effects were produced as a result of very rapid absorption, either through wounds or thin places in the bark, and a very close similarity need not be expected. It would appear that up to a certain limit the slower the injury and the longer the killed bark remains in contact with the moist earth the darker and more friable it becomes. It is possible, therefore, that if the injury in the Colorado orchards under consideration has been slowly developing on old trees for a considerable number of years this would account for the difference. As for the small and highly colored fruit, the yellowish foliage, and the restricted terminal growth, it is well known to horticulturists that these conditions may follow a nearly complete girdling of the crowns from any cause whatsoever. The purplish color of the foliage noted in some cases (Table X) late in the season both on our experimental trees and in some of the Colorado orchards is not restricted to arsenical injury. At the time it appeared in our treated trees it could be found in untreated ones where small branches had been partly broken down or the bark scraped off by farm machinery.

Realizing the difficulty of making comparisons of symptoms from written descriptions and the importance of such studies where the etiology of a disease is unknown, we made, in 1910, a rather extended trip into the affected districts of Colorado and Utah. On this trip we received most courteous consideration and many helpful suggestions from members of the Experiment Station staffs in these two States, especially from Headden and Ball. We found that the extent of injury suffered by the orchards was not overstated in Headden's bulletins and that the symptoms were most accurately described.

One additional feature impressed us very much—namely, the amount of injury done the aerial portions of the trees relative to the size of the corroded area on the crown. To be sure, many trees were entirely girdled and others nearly so, but some trees 8 to 12 inches in diameter had a dead patch of bark on the crown no larger than a man's hand and yet showed in fruit, foliage, and terminal growth a degree of injury wholly unexpected if one judges by the trees, frequently encountered, that had the crowns more or less injured by mice, blight, or other agencies. We dug up a few of the sick trees completely and uncovered the crowns and upper roots of a great many others. We did not dig up all, as this would have been a herculean task which time did not permit. The only possibility that some of the trees recorded as having only a small dead patch on the crown could have had other injuries is that such injuries were on the deeper roots below the attachment to the crown. However,

in no case of trees entirely removed was this region affected except in those having the upper portion of the crown extensively killed and the dead area extending down underneath. This disproportionate injury of crown and branches suggested a systemic poisoning in addition to the effect brought about by girdling.

A considerable search was made for evidence of parasites. Repeated examinations were made with a good traveling microscope, and material for cultures was taken back to the laboratory at Bozeman. A very small proportion of the crowns was found to be attacked by the blight organism *Bacillus amylovorus*, but these were easily distinguished from the others by field observation. A few cases differed from the blight and from the crown injury more commonly found in that the dead bark was tougher, juicier, and a more red-brown in color with a distinctly stratified appearance. By microscopic examination no microorganisms were found in these cases, and cultures from the inner bark yielded nothing. Our efforts to find parasitic fungi or bacteria in the more common form of the trouble, either by microscopical examination or by culture, yielded no positive results, though some saprophytes were found, as might be expected. We would, however, warn the reader against concluding from this evidence that the possibility of a parasitic agent was eliminated, for all experienced plant pathologists are aware that much more extended work would be required to justify such a contention.

Our observations on this trip brought no evidence against the arsenical theory. On the other hand, the contention of Ball and others (1, 2) that this trouble is caused by alkali and seepage water appeared wholly untenable, as many of the trees were in positions where such conditions were absent and there seemed to be no especial prevalence of crown injury where they occurred.

It is apparent from Ball's publications that he was confusing two troubles sharply separated by Headden, who alleged them to be due, respectively, to arsenical poisoning and to an excess of nitrates in the soil (6, 8, 9). That the latter may be due to alkali and seepage is debatable, but that is not within the scope of this paper.

We have established conclusively that arsenical compounds used as insecticides can be made to injure the crowns of trees under conditions very similar to those that result from some orchard practices. Whether or not such compounds are responsible for killing the trees under discussion in Colorado or elsewhere, or any portion of them, is quite a different question, and each reader will form his own opinions from the evidence.

We wish here to emphasize the importance of making further studies to eliminate or establish other factors as causative agents for this trouble, especially parasitic organisms and climatic conditions. The former possibility has been hardly touched so far as Colorado is concerned, though many brief studies of forms of "collar-rot" and "crownrot"

have been made elsewhere, with varying results. The latter possibility has received some consideration from Grossenbacher (3, 4) especially, but we are still left in doubt as to the application of his work to conditions in the Rocky Mountain region.

On the part of some investigators of crown injuries of fruit trees, there seems to be a tendency to take a very narrow view of the field and to ascribe to some one cause a number of troubles characterized by injury to the bark on or near the crown. That such conditions are caused by different agencies is well established, and it seems highly improbable that we will make much headway by trying to discredit the work of others in support of a narrow hobby.

CONCLUSIONS

(1) The periderm on the smooth bark of apple trees is practically impervious to arsenical solutions.

(2) If such solutions are admitted during the growing season, more or less injury will follow.

(3) The solutions may be admitted through wounds, lenticels, or latent buds.

(4) As the trees become older and rough bark is produced, the cracks made in its production will admit arsenical solutions to the inner tissues, which will be injured.

(5) Roots and branches of equal size and with similar bark are about equally susceptible to arsenical injury.

(6) If the injury is rapid, for example, the arsenic gaining entrance through a wound in the smooth bark, very definite longitudinal streaks will be produced in the bark and sapwood.

(7) If the injury is slow, the arsenic gradually entering through cracks of the normal rough bark, such streaks are very little in evidence.

(8) Paint applied to fresh wounds offers a partial, but not complete protection.

(9) Of the arsenical compounds used as insecticides, calcium arsenite is the most injurious when admitted to the inner bark.

(10) The injury is not necessarily due to impurities in the arsenical compounds.

LITERATURE CITED

(1) Ball, E. D.
1909. Is arsenical spraying killing our fruit trees? *In* Jour. Econ. Ent., v. 2, no. 2, p. 142–148.

(2) ——— Titus, E. G., and Greaves, J. E.
1910. The season's work on arsenical poisoning of fruit trees. *In* Jour. Econ. Ent., v. 3, no. 2, p. 187–197, pl. 12–16.

(3) Grossenbacher, J. G.
1909. Crown rot, arsenical poisoning and winter injury. New York State Agr. Exp. Sta. Tech. Bul. 12, p. 370–411, 8 pl. Bibliography, p. 409–411.

(4) ———
1912. Crown rot of fruit trees: Field studies. New York State Agr. Exp. Sta. Tech. Bul. 23, 59 p., 23 pl.

(5) HEADDEN, W. P.
1908. Arsenical poisoning of fruit trees. Colo. Agr. Exp. Sta. Bul. 131, 27 p., 7 pl.
(6) ———
1910. Arsenical poisoning of fruit trees. Colo. Agr. Exp. Sta. Bul. 157, 56 p., illus.
(7) ———
1910. The fixation of nitrogen in some Colorado soils. Colo. Agr. Exp. Sta. Bul. 155, 48 p., illus.
(8) ———
1910. Nitrates in the soil. Colo. Agr. Exp. Sta. Bul. 160, 8 p.
(9) ———
1911. The fixation of nitrogen in some Colorado soils. A further study. Colo. Agr. Exp. Sta. Bul. 178, 96 p., 6 pl.
(10) SWINGLE, D. B., and MORRIS, H. E.
1910. A preliminary report on the effects of arsenical compounds upon apple trees. *In* Phytopathology, v. 1, no. 3, p. 79–93, pl. 16–17. Bibliographical footnotes.
(11) WHIPPLE, O. B.
1907. Western Slope fruit investigation, 1906. Colo. Agr. Exp. Sta. Bul. 118, 16 p.

PLATE 79

Funnel used in the treatment of trunks with arsenical solutions and suspensions. × ⅙.

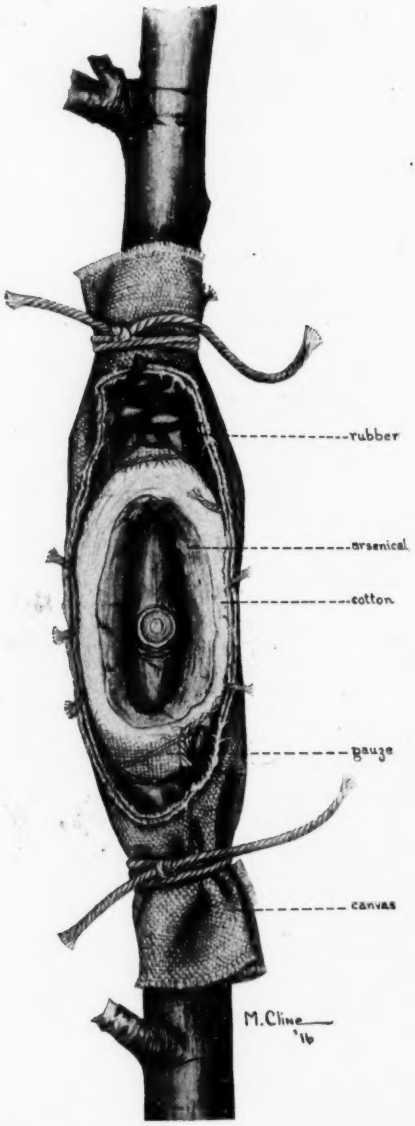

rubber
arsenical
cotton
gauze
canvas
M. Cline
'16

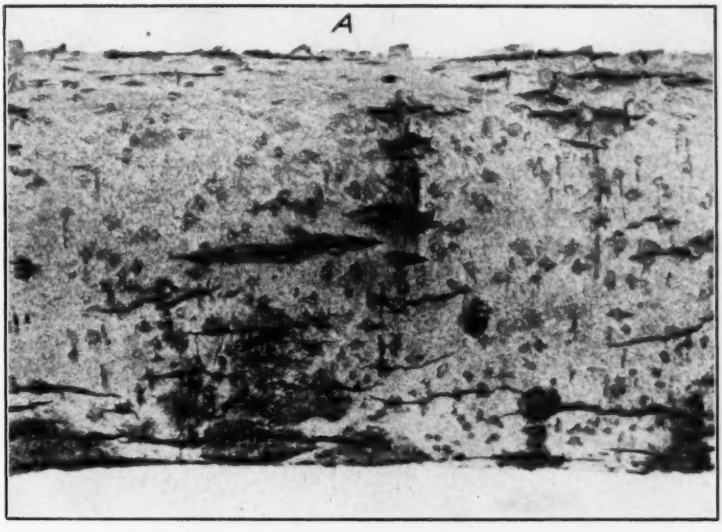
PLATE 80

Dissection of bandage used in the treatment of branches with insoluble arsenical compounds. Those used in treatment with soluble compounds were the same, except that the chemical was in solution and absorbed by the cotton. × ⅗.

PLATE 81

Character of rough bark of apple tree.

A.—First splits in the formation of rough bark. × 2/3.
B.—Roughened condition as found on practically all old trunks and limbs. × 2/3.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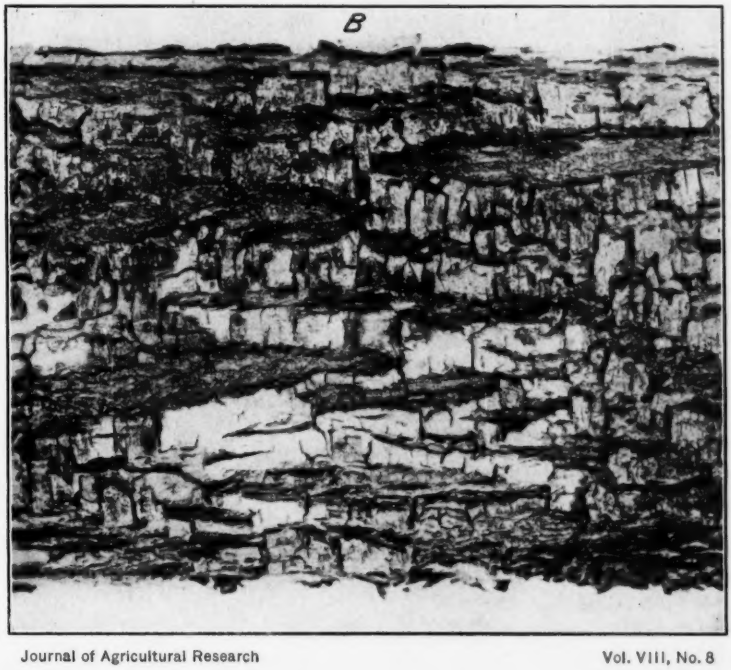
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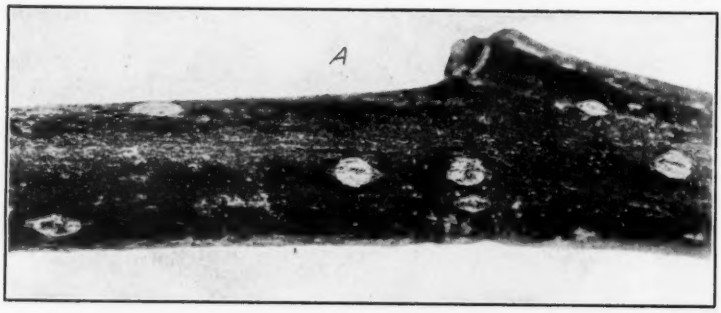
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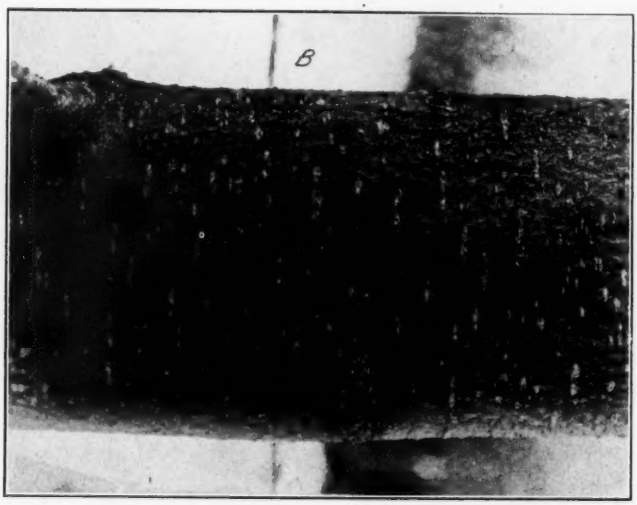
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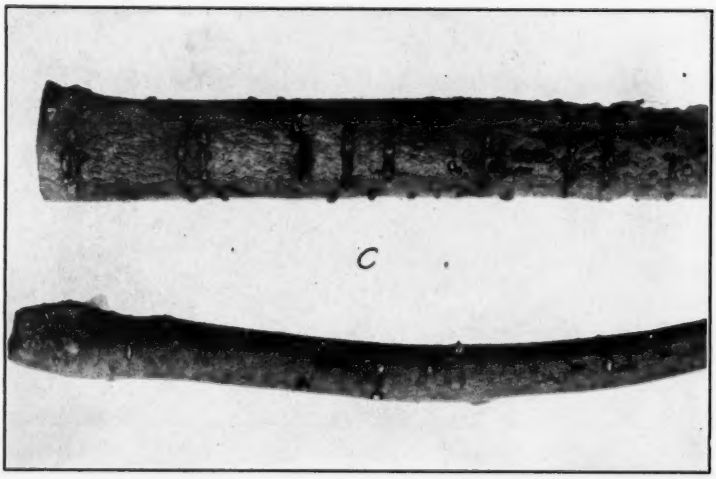

C

PLATE 82

Lenticels of different ages.

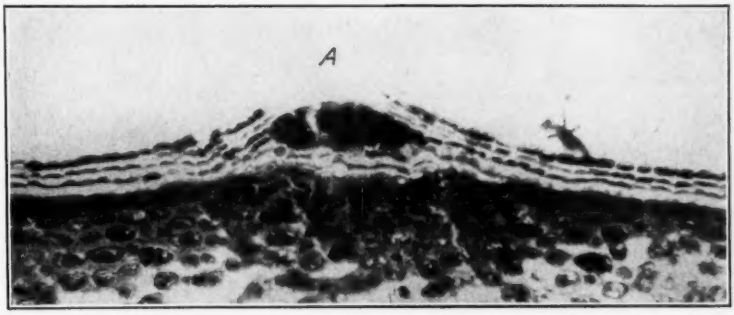
A.—Lenticels on a young shoot that has just completed the season's growth. × 6.
B.—Lenticels on a limb about 8 years old. × ⅔.
C.—Lenticels on roots. × 1.

PLATE 83

A.—Section through a lenticel just forming. × 80.

B.—Section through a well-developed lenticel. × 80.

C.—Lenticel spots formed by the absorption of zinc arsenite through lenticels inclosed in arsenical bandages. × 1.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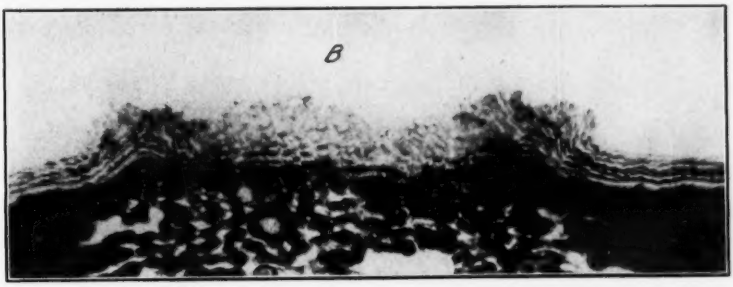
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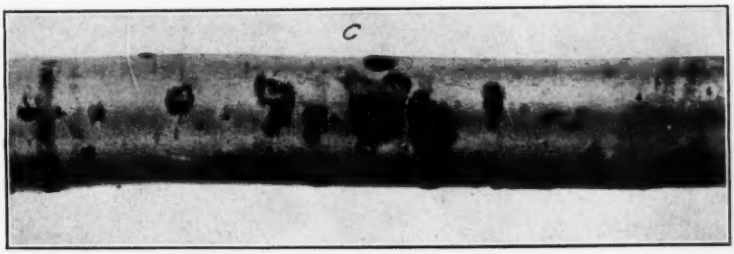
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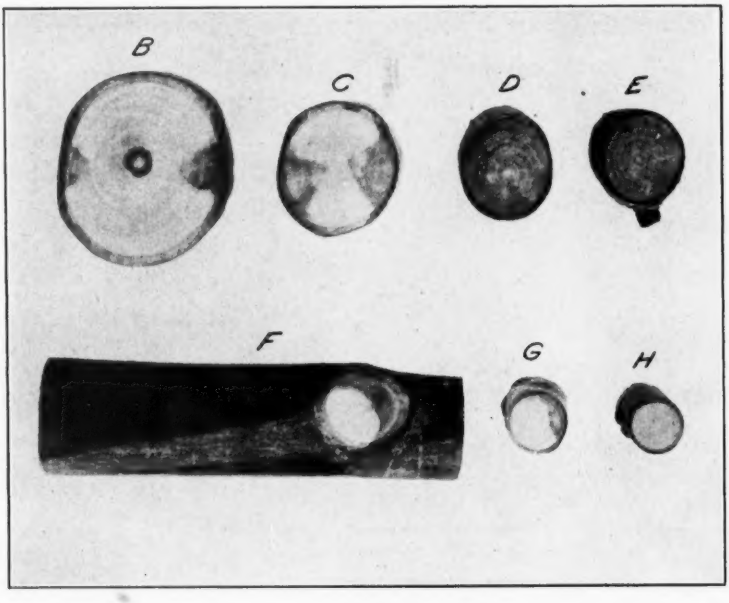
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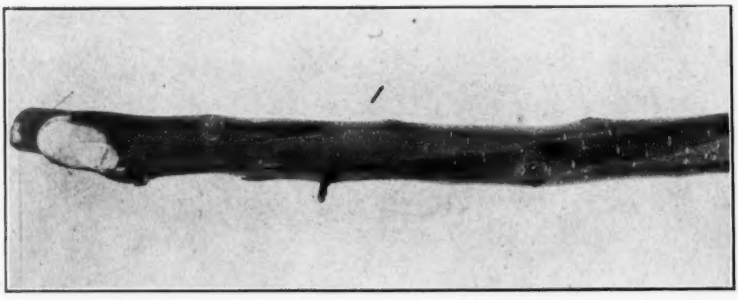
I

PLATE 84

Character of arsenical injury.

A.—Branch treated with a solution of arsenic acid. The portion photographed was inclosed by the bandage. Bark removed for photograph. The knot in the dark, injured streak was made by cutting off a limb just before the solution was applied. The other knots shown in the figure were old and showed no arsenical injury. × 1.

B to I.—Portions of tree injured by a single treatment with sodium-arsenite solution. There were two fresh wounds under the bandage on opposite sides of the limb.

B.—Cross section of main limb 1 foot below intersection with treated branch.

C.—Cross section 16 inches below bandage and just above main limb. × 1.

D.—Cross section under bandage just below wounds. × 1.

E.—Cross section 1 foot above bandage. × 1.

F.—Surface view 8 to 11 inches below bandage. × 1.

G and H.—Cross sections of side branch that joined treated branch 8 inches below bandage. The wound made by its removal is shown in figure F. Figure G is 1 inch and figure H is 6 inches above the intersection with the treated branch. × 1.

I.—Main limb with treated branch removed. The wound made by its removal is shown near the left end of the figure. × ¼.